Attachment 1

Version with markings to show changes made:

17. (Amended) An assay method for detecting a predetermined nucleic acid sequence in a sample, comprising:

a) providing a sample containing nucleic acids, and a single stranded oligonucleotide probe having a sequence complementary to the predetermined nucleic acid sequence to be detected, the single stranded oligonucleotide probe comprising at least 10 bases, wherein the first base of the single stranded oligonucleotide probe is conjugated to a first fluorescent moiety and the second base of the single stranded oligonucleotide probe is conjugated to a second fluorescent moiety, the first and second bases being positioned so that upon excitation of the first fluorescent moiety, the excitation energy is transferred to the second fluorescent moiety, whereupon the second fluorescent moiety fluoresces with peak fluorescence at a wavelength which differs from the fluorescent peak of the first fluorescent moiety;

b) contacting the sample containing nucleic acids with the single stranded oligonucleotide probe, under conditions where complementary single stranded nucleic acids hybridize, and unhybridized single stranded oligonucleotides are hydrolytically digested; and

c) measuring fluorescent transfer between the first fluorescent moiety and the second fluorescent moiety of the single stranded oligonucleotide probe of step a), subsequent to step b).

REMARKS

Rejection Under 35 USC 103(a)

The rejection of Claims 1, 2, 5-11, 15, 17, and 18 under 35 USC 103(a) as being unpatentable over Turnbow et al., in view of Holmstrom et al. and further in view of Parkhurst et al. has been maintained. Statements made by the Patent Office in response to Applicants' arguments suggest that the Patent Office does not fully appreciate the pertinent aspects of the present invention and how these pertinent aspects significantly differ from the teachings of the cited prior art. To restate, the present invention relates to the use of an oligonucleotide probe designed to specifically identify the presence of a target nucleic acid within a population of nucleic acids, through hybridization of the probe to the target nucleic acid, with subsequent nuclease digestion of probe which is not hybridized to the target nucleic acid. Said digestion modifies probe so as to eliminate detection of any remaining probe which is not in a hybrid with target nucleic acid (remaining probe includes self-hybridized probe, and probe which is hybridized to non-target nucleic acid). Importantly, probe which is hybridized to itself or to non-target nucleic acid, and thus is partially protected from nuclease digestion, is cleaved at the sites of base pair mismatch. Due to the unique design of the probe, discussed below, such cleavage completely eliminates detection of the probe. This aspect is a critical difference from hybridization based methods of detecting a target nucleic acid existing in the prior art. Such methods do not eliminate detection of partially hybridized probe (e.g., probe which is hybridized to non-target nucleic acid) and thus necessitates a size fractionation step to quantitate intact probe.

Critical to the method is the probe design. The probe necessarily comprises two different modifications. The probe of Claims 1, 2, 5-11, and 15 has a first modification which is a capture group, and a second modification which is a detectable

label. The probe of Claims 17 and 18 has two modifications which are different fluorescent moieties, wherein the first moiety produces a fluorescence which transfers to the second moiety. The first and second modifications of the probe described in all pending claims are necessarily located at positions distal to one another on the probe (e.g., they are separated by several nucleic acids). In the methods, both modifications must be present on an individual probe for probe detection, thus detection automatically verifies the complete integrity of the probe between these modifications. Importantly, any minor degradation of the probe at, or between these modifications will serve to separate the modifications, thus preventing detection of any probe fragments generated by nuclease digestion.

Elimination of the ability to detect probe which is not hybridized to target and thus not completely intact, in a hybridization detection assay, differs significantly from the cited art of Turnbow et al. which teaches an RNAse protection assay using a probe modified at only a single end, and the cited art of Holmstrom et al. which teaches detection of a PCR amplification product that contains a modification at one end, and is also labeled throughout the length of the oligo. This also differs significantly from Parkhurst et al., who teaches use of an oligonucleotide which is labeled at one end with a donor chromophore, and the other end with an acceptor chromophore, wherein the energy transfer between the two chromophores is monitored to detect changes in the conformation of the oligonucleotide in solution. In the present invention, only probe which is completely hybridized to target (each base of the probe is in a Watson-Crick base pair bond with a base of the target, such that there are no gaps in hybridization and the hybridization runs the full length of the probe) is protected from any degradation by nuclease, and thus only probe which is completely hybridized with the target is detected. A combination of the cited prior art does not produce a method of detection wherein the complete integrity of the probe is critical for probe

detection and said integrity is maintained only through hybridization with target.

Specific statements made by the Patent Office in response to Applicants' previously presented arguments for nonobviousness of the invention indicate that the arguments were not fully appreciated. Applicants would like to address these statements in an effort to further clarify the arguments. In response to Applicants' arguments of nonobviousness, the Patent Office states:

> Applicant argues that the invention improves upon the prior art since detection of partial hybridization is eliminated, thereby eliminating the requirement for gel electrophoresis. This argument is not found persuasive for several reasons. First this argument assumes that incomplete digestion is a problem which is solved by the claimed invention. There are no limitations in the claims, nor evidence of a comparison in the specification between the invention and the prior art, which support the idea that this invention teaches elimination of partial hybridization.

Applicants agree with the assertion by the Patent Office that there is no evidence that the invention teaches elimination of partial hybridization. More accurately, the present invention teaches elimination of the detection of probe which is partially hybridized to non-target nucleic acid. Contrary to the above quoted passage, Applicants' arguments do not assume that incomplete digestion is a problem with the prior art. To clarify, detection of "partial hybridization", by the methods of the prior art, does not necessarily result from incomplete digestion of single stranded probe. Complete digestion of probe which is in a hybrid with non-target nucleic acid can result in the production of detectable, albeit shortened probe. (The probe fragments which remains were not susceptible to nuclease digestion because they was in fact in a hybrid). The cited prior art Turnbow et al. teaches use of a probe which is labeled at one end, to detect a nucleic acid target by hybridizing to the nucleic acid target, subjecting the hybrids to digestion with

RNAse, and detecting the remaining probe via the attached label. It is well known in the art that this method of detecting target nucleic acid suffers from the drawback of producing detectable probe that has been truncated in the digestion process due to "partial hybridization." The "partial hybridization" results from a hybrid being formed with a nucleic acid that is not 100% complementary to the probe. This can occur via hybridization with a nucleic acid which is shorter than the probe, or alternatively that has gaps in complementarity, or by probe hybridizing to itself. Such partial hybridization provides incomplete protection in the RNAse protection assay, and can result in the production of a shortened probe which retains label, and is thus detectable by the method of Turnbow et al. Such a shortened probe is detected as background that can only be distinguished from an intact, full length probe by size fractionation. The teaching of Turnbow et al. concurs that there is a need to size fractionate the probe following digestion, in order to separate out background and artifactual bands, reciting:

> The gel separation of protected riboprobe-mRNA hybrids from any remaining labeled RNA decreases background, which permits at least a tenfold increase in sensitivity compared with a Northern blot. (page 267, first column, last sentence of first paragraph)

and:

> By digesting biotinylated probes in the absence of mRNA and by using an RNA ladder to determine molecular weights, target mRNA bands are distinguished from artifactual bands. (page 268, third column, last sentence of second paragraph.)

These statements made in Turnbow et al. clearly support the assertion that methods known in the art are not conducive to simple detection of probe remaining after RNAse digestion to accurately quantitate the amount of target nucleic acid present in the sample.

In response to Applicants' arguments of nonobviousness, the Patent Office further states:

> With regard to the Parkhurst reference, in response to applicants' arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *in re Merck & Co.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986)

Applicants respectfully point out that in our previous Amendment the Parkhurst et al. disclosure, as well as the Turnbow et al. and Holmstrom et al. disclosures, are each individually discussed with respect to their deficiencies (specifically with respect to a lack of motivation by one of skill in the art to combine the teachings of said disclosures to produce the present invention). This is not comparable to the prosecution in *In re Keller*, cited as support for the Patent Office's assertions. In *In re Keller*, appellants' rebuttal evidence to an obviousness rejection of their claims consisted solely of an affidavit which specifically discussed only a single reference article (Walsh et al.) cited in the rejection. Further, Applicants' discussion of the cited prior art is not comparable to *In re Merck & Co.*, also cited as support for the Patent Office's assertion. In *In re Merck & Co.*, a single prior art reference (Peterson) was discussed in rebuttal of an obviousness rejection as teaching away from appellants' invention.

In prosecution of the instant application, Applicants' discussion of the cited prior art does not constitute attacking the references individually, rather it constitutes discussing the teachings of each reference in order to more clearly explain why the obviousness rejection was inappropriate.

In the rejections made and maintained by the Patent Office, specific quotes from the cited prior art are taken out of context and inappropriately applied to the present invention in order to make the argument that one of average skill in the art would be motivated to combine the teachings of the three disclosures to produce the present invention. For example, Parkhurst et al. is cited as teaching a double labeled oligonucleotide for use in

signaling hybridization. However, closer examination of the teachings of Parkhurst et al. reveals that the method is not relevant to the present invention. More specifically, the detection of the hybrid is by a method whereby energy transfer from a first label, on an oligonucleotide, to a second label, on the same oligonucleotide, is less efficient when the oligonucleotide is bound in a DNA duplex hybrid, and this difference in energy transfer is used to detect differences in probe conformation which result from hybridization of the probe in a DNA duplex. The type of hybrid being detected is not necessarily the result of 100% complementarity. The term "exquisite sensitivity", as used in Parkhurst et al., is quoted by the Patent Office as describing the method of Parkhurst et al. in detecting hybridization, as motivation to combine Parkhurst et al. with the teachings of Turnbow et al. and Holmstrom et al. However, as used in the teachings of Parkhurst et al., the term "exquisite sensitivity" describes the detection of subtle wavelength changes that result from the oligonucleotide passing through different conformations (Page 292, last line). This is not a method relevant to the present invention, which teaches a double labeled oligonucleotide probe to signal the presence of an intact oligonucleotide following hybridization and nuclease digestion. Neither Parkhurst, et al., Holmstrom et al., nor Turnbow et al. teach or suggest a method of detection wherein the complete integrity of the probe is critical for probe detection and said integrity is maintained only through hybridization with target. Due to the disparity in the teachings of the cited prior art, one of skill in the art would not be motivated to produce the present invention from the combined teachings of Turnbow et al., Holmstrom, et al. and Parkhurst et al.

Absent misinterpretation of the teachings of the cited prior art, no motivation exists to combine the teachings of Turnbow et al., with the teachings of Holmstrom et al. and the teachings of Parkhurst et al., to produce the present invention, other than in an instance of hindsight reconstruction. A rejection based on

such reasoning is inappropriate. *In re Mahurkar Patent Litigation*, 28 USPQ2d 1801 (N.D. Ill 1993), the court stated as follows:

> decomposing an invention into its constituent elements, finding each element in the prior art, and then claiming that it is easy to reassemble these elements into the invention, is a forbidden ex post analysis.

In *In re Fritch*, 23 USPQ2d 1780 (Fed. Cir. 1992), the Court of Appeals for the Federal Circuit noted:

> Here the Examiner relied upon hindsight to arrive at the determination of obviousness. It is impermissible to use the claimed invention as an instruction manual or "template" to piece together the teachings of the prior art so that the claimed invention is rendered obvious. This court has previously stated that "[o]ne cannot use hindsight reconstruction to pick and choose among isolated disclosures in the prior art to deprecate the claimed invention."

Applicants are of the opinion that the above rejection has been made and maintained in error and request reconsideration of the subject patent application.

Remaining Rejections:

Claims 3 and 4 have been rejected under 35 USC 103 (a) as being unpatentable over the above referenced disclosures, and further in view of Thompson et al. and further in view of Mayrand et al. It is respectfully submitted that this rejection is obviated by the above arguments. Neither Thompson et al., nor Mayrand et al., disclose methods that make obvious the primary advancement of the present invention described above.

Claim 16 has been rejected under 35 USC 103(a) as being unpatentable over Turnbow et al., in view of Holmstrom et al., and further in view of Parkhurst et al., and further in view of Dower et al. Furthermore, with respect to Dower et al. the Patent Office stated that:

> Dower states "For instance, one could read the tag directly from the bead by sequencing or hybridization

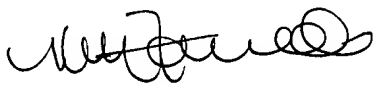
(column 19, line 47-48)". This express teaching motivates the use of hybridization detection methods such as the RNAse or S1 nuclease protection methods disclosed.

This rejection is respectfully traversed for reasons discussed above in connection with the rejection of Claims 1, 2, 5-11, 15, 17, and 18 under 35 USC 103(a) in light of the arguments made above regarding Turnbow et al., Holmstrom et al., and Parkhurst et al. The cited Dower et al. reference offers no teachings which cure the deficiencies noted above in connection with these previous rejections.

Summary

In light of the above amendment and remarks, reconsideration of the subject patent application is respectfully requested. Any deficiency or overpayment should be charged or credited to Deposit Account No. 06-0130.

Respectfully submitted,



Kevin M. Farrell
Attorney for Applicants
Registration No. 35,505
(207) 363-0558

York Harbor, ME
Dated: 7/16/02

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